CHEMICAL CHANGES IN WHEAT RELATED TO GERM DETERIORATION

by

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TABLE OF CONTENTS

INTRODUCTION.....1

REVIEW OF THE LITERATURE.....2

The Relationship of Fungi to "Sick" Wheat.....2

Chemical Changes Occurring in Wheat During Storage and Deterioration.....5

Other Characteristics of the Browning Reaction.....8

Spectrophotometric Characteristics of Protein and Protein Split Products.....9

Pigments and Fluorescent Substances Present in Wheat and Wheat Germ.....10

Conclusions from Literature Survey.....12

MATERIALS.....12

EXPERIMENTAL.....13

Preliminary Spectrophotometric Studies of Extracts of Sound and Germ-damaged Wheat.....13

Wheat Germ Storage Studies.....15

Spectrophotometric Characteristics of Extracts of Germs Dissected from Germ-damaged and Sound Commercial Samples.....18

Fluorescence Studies on Wheat Germ and Ground Wheat Meal.....18

Role of Vitamins in Fluorescence of Aqueous Germ Extracts.....26

Relationship of Fluorescence to Optical Density.....29

Chromatographic Separation of the Dark Pigment from Aqueous Extracts of Brown Germ...31

Fluorometric Method for Germ-damaged Wheat.....32

Fluorometric Characteristics of Extracts of Germ and Non-germ Portions of Damaged and Sound Wheat.....37

Correlation of Commercial Grain Inspection with the Fluorometric Test.....38

Fluorometric Studies of Different Classes and Varieties of Sound Wheat from Various Locations.....39

DISCUSSION AND CONCLUSION.....45

SUMMARY.....50

ACKNOWLEDGMENTS.....53

LITERATURE CITED.....54

APPENDIX.....58

INTRODUCTION

A type of damage in stored wheat occurring in both terminal and country elevators and known in the trade as "sick" wheat has been recognized since about 1921 (6). "Sick" kernels develop deadened germ ends, easily distinguished as damaged by their black or brownish appearance when the germ is exposed by scraping off the bran layer on top of the germ. Federal and licensed grain inspectors count the number of damaged kernels determined in this manner, in approximately ten grams of grain, and calculate the percentage of damage. This type of damage is sometimes accompanied by a musty odor. "Sick" wheat has also been defined as that type of deteriorated wheat in which the seed loses its viability and the germ darkens in color and becomes rancid. Under extreme conditions the germ may be completely destroyed leaving only a cavity in the grain.

It is apparent that the subjective method used at present by grain grading inspectors for determining the percentage of "sick" wheat is highly unreliable and inadequate, since evaluation by visual inspection of the kernels is by no means trustworthy and varies from one individual to another. It was primarily for this reason that the present research was undertaken. The overall practical objective of this work was to find some precise method of measuring the amount of damage in a sample of "sick" wheat more quantitatively than that used by the grain inspectors. The availability of such a test would permit more informative

experimental studies to determine the factors causing the "sick" wheat condition.

Before such a method could be devised, however, it was necessary to investigate differences in the physical and chemical properties between germ-damaged and sound wheat. One major approach in the present study was an investigation of the physical and chemical properties of the dark pigment present in the germ of "sick" wheat.

The two phases of study described herein include a qualitative study of dark pigment of the wheat germ and an initial step toward devising a quantitative method for determining the degree of deterioration in a "sick" wheat sample. The work involved an investigation of the spectral and fluorometric characteristics of wheat and germ extracts and possible relationship of these properties to the vitamin constituents of the grain. The fluorometric study was extended to determine the potentialities of this technique as an objective method for the quantitative determination of damage related to the "sick" condition.

REVIEW OF THE LITERATURE

The Relationship of Fungi to "Sick" Wheat

The cause of "sick" wheat as distinct from causes of other forms of damage of high-moisture wheat in storage, such as heating, is not definitely known. The condition seems to develop principally in wheat stored in large quantities, and makes its appearance

only after a considerable time. It is sometimes found, however, in freshly harvested wheat when delivered to elevators for storage. Its occurrence may be associated with conditions favoring germination followed by conditions which kill the germ. Considerable evidence is accumulating that mold growth is a major cause of preliminary deterioration leading to the darkened germ. In the study of factors which are associated with damage in stored wheat, Swanson (29) found, even when mold growth was inhibited by exclusion of air from the grain, or by treatment of the seeds with ethyl mercury phosphate, "sick" wheat would develop in the stored grain with time, and that under such conditions serious damage to baking quality of the flour did take place.

Thomas (33) and Tervet (32) found that certain species of fungi decrease seed viability through some toxic principles they elaborate. Thomas made germination tests on wheat after treating the seed with filtrates of pure cultures of 13 species and strains of molds which are commonly found on grain. While all of the filtrates somewhat lowered the germination, Aspergillus flavus had a very pronounced effect. Working with soybeans, Tervet (32) found that an increase in the moisture content and time of storage at room temperature resulted in an increase in the number of mold-infected seeds and a decrease in the germination. Treatment of the soybean seeds with cell-free filtrates of three species of molds, Aspergillus flavus, A. niger, and A. ochraceus, lowered the germination.

"Sick" wheat was produced in the laboratory by Carter and

Young (5). They stored wheat in sealed containers at various moisture contents and temperatures over time intervals up to 687 days and followed changes in fat acidity and decrease in germination. They eliminated all samples on which molds were visible and concluded that "sick" wheat formation is associated with anaerobic storage. In general, an increase in "sick" kernels, favored by high moisture and temperatures, was accompanied by an increase in fat acidity and a decrease in germination.

The various types of microflora which occur on commercial samples of "sick" wheat were investigated by Milner, Christensen, and Geddes (21). They also studied the influence of temperature, moisture, time, and storage atmosphere, on the development of "sick" wheat in the laboratory. Germ-damaged kernels hand-picked by federal grain inspectors from commercial lots of wheat were found to be infested, invariably, with fungi, principally A. glaucus, Penicillium and, to a lesser extent, with A. candidus, A. flavus and A. niger. In contrast, sound wheat samples from the same lots were largely contaminated with Alternaria which disappeared when storage conditions favored the proliferation of the Aspergilli. When sound wheat was inoculated with various molds and bacteria, isolated from "sick" wheat, and stored in air, it lost viability faster than did the controls, and most of the nonviable kernels had symptoms typical of "sick" wheat. "Sick" kernels were produced by storing wheat containing 18 per cent moisture under atmospheres of carbon dioxide, nitrogen, or oxygen, in sealed containers. Only under oxygen did molds proliferate, where-

as "sick" kernels appeared under all atmospheres.

In recent unpublished studies, Christensen[1] found a good correlation between the increase in certain storage molds on and in wheat and the production of "sick" wheat. He inoculated wheat heavily with storage molds and stored it for 10 to 15 days at moisture contents of 18 to 21 per cent, then dried the grain to 14 per cent and stored it for 2 months. During this 2 months of storage, a high percentage of "sick" wheat developed. Wheat free of storage molds, except for some Aspergillus glaucus similarly stored, developed almost no "sick" wheat. Christensen pointed out that it seems questionable whether, in actual stored grain, factors other than storage molds ever play much part in the development of "sick" wheat.

Thus, the evidence reviewed indicates that the development of "sick" wheat can occur under anaerobic storage conditions. Under aerobic conditions, the deteriorative effects of molds increase its formation. It is unlikely that bacteria are an important contributory factor since they grow only at moisture contents equivalent to a relative humidity of about 95 per cent.

Chemical Changes Occurring in Wheat During Storage and Deterioration

Zeleny (38) reported that the rate of chemical changes in stored grains depends on the conditions of storage such as time,

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1 Clyde M. Christensen, "Studies on 'Sick' Wheat", Progress Report No. 2, Nov. 1, 1952.

temperature, etc., and that the following transformations are found to occur in the components of stored grain: (1) the starch is attacked by alpha and beta amylases, converting it to dextrins and maltose; (2) proteins are hydrolyzed by proteolytic enzymes into polypeptides and, finally, into amino acids; and (3) changes in the fats may be oxidative, resulting in rancid flavor and odors, or hydrolytic resulting in free fatty acids.

Pertinent in connection with the darkening which develops in "sick" wheat kernels are the data obtained by Johnson and Hagborg (17). They found that at high temperatures, especially when combined with a high humidity, melanistic areas may develop on the glumes, lemmas, peduncles, and internodes of Apex and Renown wheat in the absence of any infection by pathogenic organisms. Milner et. al. (21) stated that data of Johnson and Hagborg (17) suggest that enzymic or even such nonbiological effects as interaction between carbohydrates and nitrogen-containing compounds (Maillard or "browning" phenomenon) are possible under such conditions. The darkening of "sick" wheat, which appears first in the embryo portion may be due to such a type of reaction (30, 31, 35, 36, 37).

Similar types of deterioration occur in a variety of foodstuffs such as dried fruits, vegetables, dried egg products, etc., and are accompanied by darkening of tissue and development of off-flavors. There are three generally recognized theories concerning the browning reaction:

1. Maillard (or melanoidin) condensation theory

2. Ascorbic acid theory

3. Active aldehyde theory

Maillard (20) contributed a considerable amount of knowledge to this field when he discovered the reaction products in the browning phenomena due to condensation of carbohydrate and protein materials. He postulated that the active groups of the sugar (carbonyl or aldehyde group) react with the amino group to form first a Schiff base. Successive decarboxylation and dehydration of this product give dark materials known as melanoidins. Water and carbon dioxide evolution accompany this reaction in the ratio of 12 moles of water to 1 mole of carbon dioxide.

Four general types of interacting condensation systems are reported to be responsible for browning. According to Danehy and Pigman (9), these types are:

1. Sugar and amino compounds other than amino acids
2. Sugars and amino acids
3. Sugars and proteins or polypeptides
4. Polysaccharides and proteins

The alpha amino group is said to be largely responsible for the reaction in the case of amino acids, but the beta group could also be involved. Other amino groups apparently do not react.

Ascorbic acid, if present, is destroyed to the extent of approximately 85 per cent. The rate of destruction of ascorbic acid is roughly proportional to the development of color and the production of carbon dioxide in dilute solutions. According to this theory, ascorbic acid and related compounds oxidize to yield

reactive products that polymerize or react with nitrogenous constituents present to form brown pigments (24).

The active aldehyde theory has been advanced by Wolfrom et. al. (36) who studied the ultraviolet absorption spectrum of glucose refluxed in distilled water. The characteristic absorption curve for one of the products 5(hydroxymethyl)-2-furfural, exhibited maxima at 228 and 285 mu, similar to browning reaction products. The interaction of amino acids and reducing sugars has been studied by Wolfrom et. al. (37). They found that xylose was, in part, transformed to 2-furfuraldehyde, while the hexose molecule was transformed to 5(hydroxymethyl)-2-furaldehyde. It was noted that the presence of significant amounts of glycine had a promoting effect on the production of the furans. Proof that 5(hydroxymethyl)-2-furaldehyde was formed from D-glucose in the presence of glycine was established by the actual isolation of this compound from such a mixture. From this, they concluded that the carbonyl-amino-reaction occurred only slightly in dilute aqueous solutions of D-glucose and glycine, and that, therefore, such interactions are not of major significance in browning.

Other Characteristics of the Browning Reaction

The brown pigments formed as a result of the browning reaction have definite characteristics. They fluoresce strongly in ultraviolet light. Browning and fluorescence develop simultaneously and their formation proceeds at parallel rates. This has been

shown to be true by Tarassuk and Simonson (31). With progressive browning, there is a decrease in pH. This decrease in pH was utilized by Frankel and Katchalsky (11) to determine the extent of interaction between aldose and alpha-amino acids.

Absorption data obtained with the spectrophotometer have been found useful by Mohammad et. al. (23) in following the rate of color development in the browning reaction. A separation of the browning products was successfully performed by Hanna and Lea (15). They reacted mixtures of glucose and acetylated lysine and found the reaction complexity to increase as the humidity was increased. Sixty per cent relative humidity was required to produce all changes of the Maillard reaction. They examined the reaction products by descending paper chromatography using 80 per cent aqueous propanol as the eluent. The precise chemical nature of these products separated was not determined, and an examination of the unheated papers under an ultraviolet lamp revealed the presence of a series of fluorescent products.

Spectrophotometric Characteristics of Protein and Protein Split Products

Since degradation of proteins by hydrolysis appears to be a factor in germ deterioration, information concerning the spectral characteristics of these materials is pertinent. The spectrophotometer has been used to a considerable extent in the ultraviolet region to characterize proteins and protein hydrolysates. For the absorption spectra of the greatest number of most proteins

there is a broad absorption band with a peak at about 280 mu and a minimum on the short wavelength side at about 250 mu (8). From quantitative studies of simple proteins, this band can be correlated with the presence of tyrosine, tryptophan, and phenylalanine. A number of proteins exhibited absorption bands at wavelengths above 280 mu that could be interpreted in terms of a non-proteinaceous chromophore. Similarly, nucleoproteins show a maximum in the region of 260 mu, which is associated with the presence of purines and pyrimidine nuclei (14). A micromethod for the estimation of degradation products of proteins based on the ultraviolet absorption of their copper salts was devised by Spies (27,28). He studied several amino acids, dipeptides, and tripeptides by this method, measuring absorption due to their copper salts at 230 and 620 mu. This method was found to be particularly suitable for studying the rate and degree of hydrolysis of proteins, such as that of casein in aqueous acid media.

Pigments and Fluorescent Substances Present in Wheat and Wheat Germ

An important phase of this work involved a spectrophotometric and fluorometric study of aqueous extracts of ground whole wheat and wheat germ. Consequently, information was needed concerning naturally occurring fluorescent and light absorbing materials present in wheat and wheat germ. The pigments of the dilute alcohol or acetone extracts of whole wheat meal were studied by Markley and Bailey (22). They found that a major group of pigments other

than carotenoids were the flavones. This group is water soluble and phenolic in character. Dilute alcohol extracted very little of the carotenoid pigments. An acetone extract of Mindum Durum wheat was unsuitable for transmittancy studies in the ultraviolet region since the solvent has a very pronounced absorption spectrum of its own. Ethanol, 67 per cent, was found to be a well suited solvent and extracts yielded absorption bands at 269 mu to 325 mu. The absorption spectrum of the same solution, made alkaline with sodium hydroxide showed no shift in the peaks but the concentration of the pigment present appeared to be quadrupled or a considerable change in the intensity of absorption occurred with change in pH. This is quite characteristic of many naturally occurring plant pigments having indicator properties among which are included the flavones.

The two water soluble vitamins which have fluorescent properties and exist in significant quantities in wheat and wheat germ are riboflavin and thiamine. Schultz et. al. (25) reported that wheat germ contains, on the average, 30.0 micrograms of thiamine per gram, and whole wheat approximately 5.7 micrograms per gram. Connor and Straub (7) found whole wheat to contain 0.89 to 2.03 micrograms per gram of riboflavin, while wheat germ contained 3.78 to 5.56 micrograms per gram. Wheat kernels containing 1.0 micrograms per gram (dry weight basis) of riboflavin and 7.0 micrograms per gram of thiamine were found to increase in these vitamins considerably upon germination as follows (4):

Wheat	Riboflavin (ug. per gm.)	Thiamine (ug. per gm.)
Dormant	1.3	7.0
Germinated	5.4	9.0

A study of the fluorescence of riboflavin with change in pH was made by Karrer and Fritzsche (19). They reported optimum fluorescence at pH of 3.8 and 7.0

Conclusions from Literature Survey

It appears from the foregoing that the "sick" wheat condition is at this time so poorly characterized in the chemical sense that one must draw on a broad background of chemical phenomena involved in similar types of spoilage in order to speculate concerning the nature of this type of deterioration.

MATERIALS

Samples of unprocessed granular wheat germ used in this work were obtained from General Mills Inc., Minneapolis, Minnesota. Approximately fifty samples of commercial wheat having varying degrees of germ damage were acquired from the Federal Grain Inspection Office, Kansas City, Missouri. These samples were composites of unknown varieties and the percentage germ damage was determined by the grain inspectors.

For the study of fluorescent properties of varieties grown in different localities, six different varieties, each grown in twelve different localities in the state of Kansas, were selected

from the Laude Environment Series.

Sound wheats studied were from the following sources and classes:

Class	Location
Soft red winter (11 varieties)	Wooster, Ohio
Pacific white (14 varieties)	Lind and Pullman, Washington
Hard red spring (2 varieties)	Langsdon Substation, North Dakota
Hard red winter (3 varieties)	Kansas State College

EXPERIMENTAL

Preliminary Spectrophotometric Studies of Extracts of Sound and Germ-damaged Wheat

The well known utility of spectrophotometric technique for evaluating chemical differences in biological materials prompted the use of this method in preliminary studies. Ground wheat, both germ-damaged and sound, was extracted with various solvents. The light absorption of these extracts was then measured in the region 220 to 350 mu using a Beckman Model DU Spectrophotometer with quartz cuvettes. The wheat samples were ground in a Wiley mill using a No. 30 mesh sieve. Then, a 5 gm sample of the ground wheat meal was extracted with 100 ml. of solvent for 1 hr, with occasional swirling approximately once every 15 minutes. Shaking the mixture proved to be undesirable since starchy materials were thrown into the suspension with consequent difficulty in filtering. At the end of 1 hour, the extraction mixture was decanted and fil-

tered through a Seitz filter using the standard type pads. Immediately following the filtration, 1 ml. of the filtrate was diluted to 5 ml with the solvent used and absorption measurements were made. The dilution was made in order to keep the optical density within the limits of the scale of the instrument.

Among the various solvents used for the extraction media were distilled water, 95 per cent ethanol, water saturated butanol, 2.5 per cent aqueous potassium hydroxide, acetone, dioxane, and other nonpolar solvents. The dark pigment in the germ of the damaged wheat appeared to be more soluble in distilled water than any of the other solvents used. The use of aqueous solvents having pH greater than 7.0, for example 2.5 per cent KOH, resulted in erratic differences in optical density. This was noted previously by Markley and Bailey (22) They found that a water soluble flavone pigment present in wheat meal possessed indicator properties at a pH greater than 7.0, yielding a yellow color. Consequently, in order to avoid erratic fluctuations in the absorption spectra of these aqueous extracts due to this natural pigment, a 0.2N aqueous hydrochloric acid solution was employed in this work for all subsequent determinations. A plot of the optical density of aqueous extracts of sound and germ-damaged wheat versus the wavelength in millimicrons is shown in Fig. 1. Differences in optical density between the germ-damaged wheat sample, approximately 50 per cent damaged, and the sound sample do exist but such differences are not great. There appears to be only a slight increase in optical density in the range of 240 to 265 mu and 295 to 350 mu in the

case of the germ-damaged wheat extract.

Wheat Germ Storage Studies

Since discoloration of the germ is the primary interest of grain inspectors in determining "sick" wheat, a direct approach toward the study of the pigment problem seemed possible through the use of fresh, untreated wheat germ. Some samples of whole germ were sealed in jars and set aside in storage at an elevated temperature, 38° C. It was found that, in the case of the whole germ, browning occurred to a visible degree after 18 days. After 35 days, visible mold growth was detectable, accompanied by an odor of mustiness. These results led to further, more quantitative studies of germ storage.

Four 300 gm samples of whole germ containing 12 and 17 per cent moisture were stored at two fairly constant temperatures of 25° C. and 38° C. These samples were placed in Kerr Mason jars and sealed with screw lid tops. The germ was further protected from the air by sealing the tops with masking tape. At regular intervals, samples were withdrawn and analyzed for fat acidity and reducing and nonreducing sugars in accordance with specifications of the Association of Official Agricultural Chemists (1).

With time in storage, there was a definite increase in fat acidity accompanied by an increase in reducing sugars, calculated as maltose. These increases appear to be accelerated with mold growth at higher temperatures and moistures as was the case of the

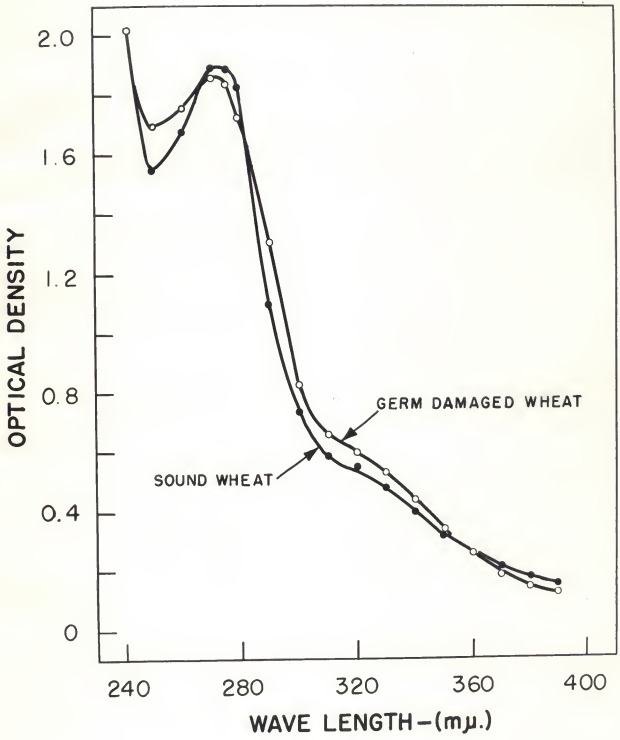

Fig. 1. Absorption spectra of aqueous extracts of sound and germ-damaged wheat.

samples stored at 38° C. and 17 per cent moisture. The results of these determinations on the stored wheat germ are listed in Table 1. Browning was found to increase in the germ with prolonged storage, elevated temperature and moisture. The rate of browning was measured as follows: Samples of wheat germ stored at 38° C. and 17 per cent moisture were taken from storage at different times and ground in the Wiley mill using the No. 30 sieve. Then, 1 gm samples of the ground germ were weighed on the analytical balance into 125 ml Erlenmeyer flasks. Fifty ml of .2N HCl was then added to each flask and the samples were extracted for 1 hour. These mixtures were swirled once every 15 minutes. At the end of the 1 hour period, they were filtered through Whatman No. 1 filter paper. A 10 ml aliquot of the filtrate was diluted to 25 ml with the 0.2N HCl solution as the diluent, and the light absorption (optical density) of these diluted extracts was measured in the region 240 to 350 mu.

The absorption curves of Fig. 2, made from extracts of the browned and fresh germ, compare closely with those obtained from extracts of sound and germ-damaged wheat, with the characteristic increases in optical density at 265 to 280 mu and 295 to 350 mu. A further examination of these regions disclosed that the wavelengths of 335 mu and 345 mu would be most useful for studying the rate of browning of wheat germ since the optical density at these wavelengths in relationship to changing concentration conformed closely to Beer's Law. This relationship was found to be linear.

Spectrophotometric Characteristics of Extracts of Germs Dissected from Germ-damaged and Sound Commercial Samples

A sample of wheat graded for germ-damaged kernel content and obtained from the Federal Grain Inspection Office in Kansas City was segregated by visual inspection into sound and germ-damaged portions. The germs were then dissected from each portion and the two germ samples obtained were ground finely in a mortar. Portions, 0.1 gm in size, of germ from sound and germ-damaged wheat were extracted for 1 hour in 15 ml of 0.2 N HCl solution and then were filtered. The same procedure for measuring the optical density as described previously was used and a plot of optical density versus wavelength was carried out.

Extracts of dissected germ from sound and germ-damaged kernels yielded curves shown in Fig. 3. These curves show that considerable differences exist in spectral characteristics of "sick" and sound germ in commercial samples. It is also to be noted that the characteristics of these curves are directly similar in shape and trend to those obtained with germ which had turned dark in laboratory storage.

Fluorescence Studies of Wheat Germ and Ground Wheat Meal

One of the characteristics of browning products formed as a result of the condensation of carbohydrates and proteins is their ability to fluoresce strongly in ultraviolet light (31). The

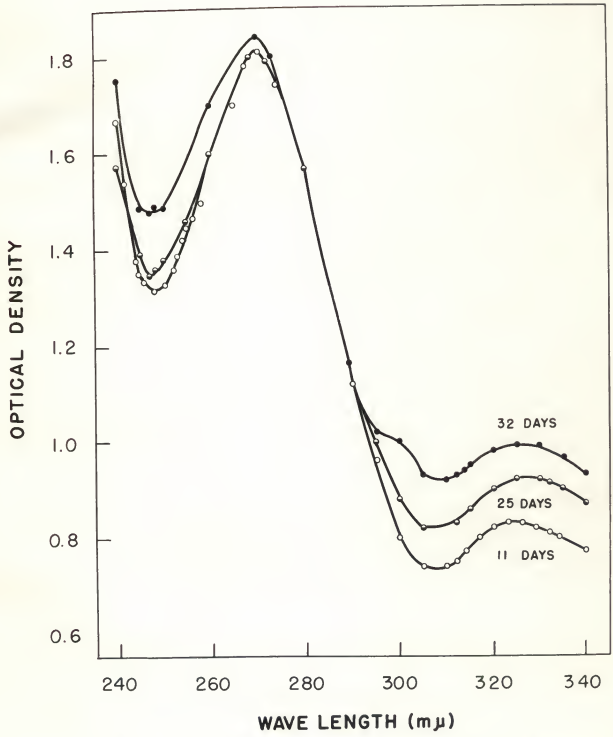

Fig. 2. Absorption spectra of 0.2 N HCl extracts of wheat germ stored at 17% moisture and 38°C. for various periods of time.

Table 1. Effect of temperature, moisture, and time on the chemical composition of whole wheat germ.

Days storage	Fat acidity[1] Per cent moisture		Total sugars[2] Per cent moisture		Reducing sugars[2] Per cent moisture		Non-reducing[2] sugars Per cent moisture		Visual appearance Per cent moisture
	12	17	12	17	12	17	12	17	17
Temperature = 25° C.									
0	128	128	295	295	166	166	129	129	No change
13	140	152	279	355	145	189	134	166	No change
23	170	191	254	276	145	406	109	170	Slightly brown
35	220	278			130	465			Slightly brown
Temperature = 38° C.									
0	128	128	295	295	166	166	129	129	No change
13	164	175	355	403	132	265	223	138	Slightly brown
23	220	230	269	463	145	135	124	328	Intensely brown
35	322	356			158	434			Intensely brown with noticeable mold growth

1 Milligrams of sodium hydroxide per 100 grams dry whole germ
2 Milligrams maltose per 10 grams germ

possibility that the dark colored substance produced in wheat germ during storage might be of such a nature and possess fluorescent properties, was investigated. This was accomplished with extracts of ground wheat meal and germ, both fresh and browned, using a Coleman Photoelectric Fluorometer, with B_1 and B_2 filters and sodium fluorescein (0.1 ppm) as a fluorescence standard. A preliminary test made on the aqueous acid extract of ground wheat and wheat germ revealed a distinct difference in the intensity of fluorescence of germ-damaged wheat and sound wheat and of germ browned in storage in comparison with sound germ. Initially, a number of solvents were used in order to find the medium most effective for extracting this fluorescent substance, since it is not known whether the brown substances and the fluorescent substances are identical. Among those solvents tried were ether, butanol, water saturated butanol, acetone, benzene, 95 per cent ethanol, dioxane, petroleum ether, distilled water, and 0.2 N hydrochloric acid. Those which extracted the fluorescent materials were water saturated butanol, alcohol, water and 0.2N HCl. Any compound which contained water to a greater or lesser degree, such as water saturated butanol and 95 per cent ethanol, extracted the material. Upon comparing fluorescence intensity of the extracts, however, the 0.2N HCl solution was found to be the most effective extraction medium.

The method of extraction was as follows. The sample of wheat germ was ground in the Wiley mill as before. A 1 gm sample was accurately weighed and extracted for 1 hour with 50 ml of 0.2N

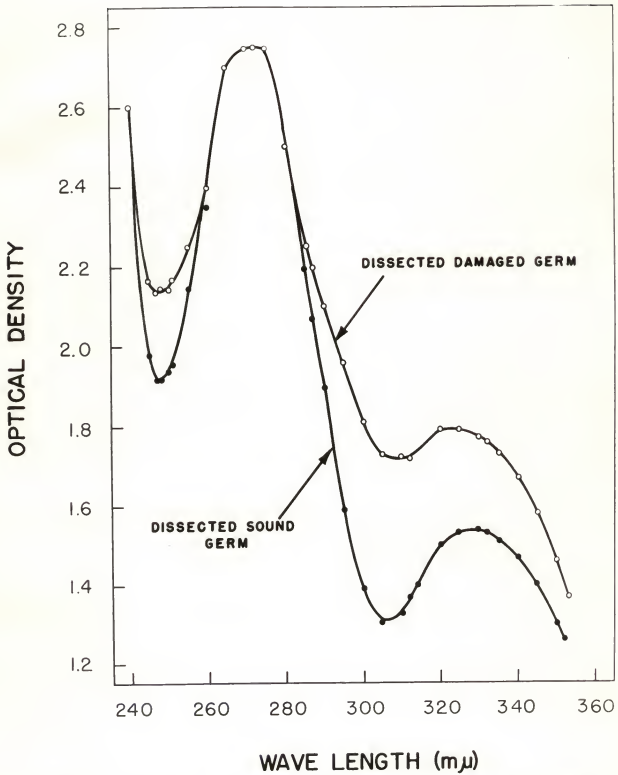

Fig. 3. Absorption spectra of 0.2 N HCl extracts of germs dissected from "sick" and sound kernels from the same sample.

HCl solution. The mixture was swirled twice during the extraction period, care being taken to avoid throwing particles into suspension which would cause difficulty in filtering. At the end of one hour, the supernatant extract was decanted through Whatman No. 1 filter paper and the clear undiluted extract was read immediately in the fluorometer using the B_2 filters with the fluorescein solution set at 30 on the scale. Later in this work, it was found by using B_1 filters instead of the B_2 the intensity of the fluorescence of these extracts was increased approximately fifty fold. Therefore, when using the B_1 filters it was necessary to dilute the extracts 1 ml to 50 ml.

The extracts of the browned germ and germ-damaged wheat were found to fluoresce with a much greater intensity than those of the sound germ and sound wheat. The following results were obtained using the methods described above:

Germ Sample	Fluorescence
Browned germ (2 months storage, 18 per cent moisture, 38° C.)	31.3 units
Sound germ	5.0 units

Calculation of total fluorescence to dry sample basis:

Browned germ	990.6 units/gm
Sound germ	133.3 units/gm
Difference	857.3 units/gm

As shown above, the extracts of the browned germ were found to fluoresce with a much greater intensity than those of the sound

germ. As a result of a 2 months storage at 18 per cent moisture and 38° C., the fluorescence of the germ increased 857.3 units/gm.

Another study made on the fluorescent material which accumulates during storage in germ was the effect of time on extraction of the fluorescent substances with 0.2N HCl and the effect of pH on the fluorescence of materials extracted. Buffers of the following concentration and composition were used (13).

Buffer	pH
Potassium chloride (0.2M) - hydrochloric acid (0.2M)	1.0 - 2.0
Potassium acid phthalate (0.2M) - hydrochloric acid (0.2M)	2.5 - 3.5
Disodium phosphate (0.1M) - monopotassium phosphate (0.1M)	4.0 - 8.5
Sodium bicarbonate (0.1M) - sodium carbonate (0.1M)	9.75 - 11.0

The measurement of fluorescence was made essentially the same as previously indicated using fluorescein as the standard and the B_2 filters. Instead of using 50 ml of the 0.2N HCl as the solvent for 1 gm of ground wheat germ, however, 50 ml of each of the above buffers were used as the extraction media. The results obtained from the study on the time of extraction of the fluorescent substances are shown in Table 2 and these indicate that they are extremely soluble in 0.2N HCl and that a one hour extraction period gives optimum results with a sample to solvent ratio of 1 gm to 50 ml. A plot of pH versus intensity of fluorescence gave the curve in Fig. 4. Two maxima occur on this curve,

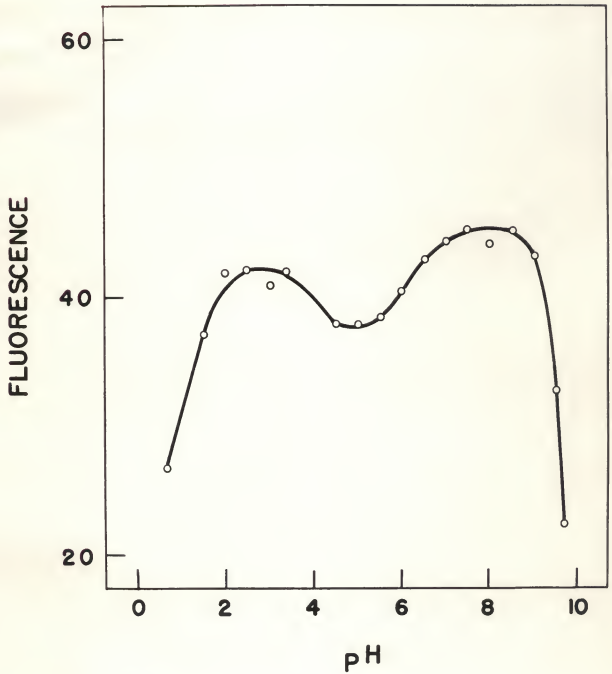

Fig. 4. Intensity of fluorescence of wheat germ extract with change in pH.

one at pH 2 to 3, and the other at pH 7 to 9.

Table 2. Effect of time of extraction of browned germ upon intensity of fluorescence using 0.2N HCl as solvent.

Extraction time in minutes	Intensity of fluorescence
5	53.0
10	55.3
15	57.0
30	59.0
45	59.0
60	59.0
120	58.5

Role of Vitamins in Fluorescence of Aqueous Germ Extracts

In the course of the studies of the fluorescence of extracts of normal and damaged wheat germ, the question arose concerning the identity of the curve relating the influence of pH on fluorescence of these extracts with that produced by riboflavin under the same conditions. It is known that in wheat during its germination an increase in riboflavin and thiamine content occurs (4). In view of this evidence, studies were made to determine if the riboflavin in wheat germ increased or decreased in quantity or remained constant during germ storage at high moisture and temperature. If the riboflavin content of the wheat germ did increase during the storage of the wheat germ and is extracted with the 0.2N HCl solvent, the difference in fluorescence between sound and brown stored germ could be attributed to this riboflavin increase. To investigate this problem, a series of riboflavin

assays were made on the germ showing varying degrees of darkening using the rapid method for riboflavin developed by Hoffer, Geddes, and Alcock (16). A striking similarity exists between the curve in Fig. 4 and the curve showing riboflavin fluorescence as a function of pH. Riboflavin fluorescence reaches a maximum at pH 3.8 and 7.0. This similarity led to the investigation of the riboflavin content of sound and darkened wheat germ. The data shown in Table 3 were obtained from assays using a group of wheat germ samples containing 18 per cent moisture and stored for the various periods of time as indicated. These results show that fluorescence increased with germ darkening but that no significant change in the content of riboflavin occurred.

Table 3. Riboflavin content of wheat germ of 18 per cent moisture after different storage periods at a constant temperature of 38° C.

Days storage	Fluorescence of extract (std. at 60)	Riboflavin content (ug/gm) dry sample basis
17	28	5.5
25	42	4.6
32	57	5.0

One other water soluble vitamin which exists to a sizeable amount in wheat germ is thiamine. Although thiamine is not fluorescent, it can be easily oxidized to thiochrome, a highly fluorescent substance. It was not known whether thiamine played a

part in the fluorescence of the browned wheat germ. Consequently, assays for thiamine were made on sound germ and darkened germ using the method of the American Association of Cereal Chemists (1) with a few modifications.

Duplicate ground samples of 0.1 gm each were extracted for 30 minutes with 50 ml of 2 per cent acetic acid-potassium chloride solution at a temperature of 70° C. At the end of this time, the samples were filtered and the filtrates were analyzed. In the fluorometric thiamine determination, the thiochrome is extracted from its aqueous medium with isobutanol. The fluorescence of the isobutanol layer then is measured. A 5 ml aliquot of the undiluted filtrate of wheat germ was made alkaline with 15 per cent NaOH and extracted with isobutanol to determine the amount of thiochrome present, if any, as a result of oxidation of thiamine during deterioration. Then, total thiamine content of wheat germ was determined by employing standard fluorometric methods for B_1 assay as outlined in Cereal Laboratory Methods (1).

In the thiamine assay, the fluorescence of the isobutanol layer on the basis of 0.1 gm of dry sample of wheat germ was as follows:

Browned germ	87.5 units
Sound germ	79.0 units

Total thiamine content of the germ was found to be as follows:

Browned germ	13.5 ug/gm
Sound germ	14.0 ug/gm

It appears that very little thiamine, if any, is converted to thiochrome during deterioration of wheat germ. The fluorescence is apparently unrelated to the water soluble vitamins, riboflavin and thiamine.

Relationship of Fluorescence to Optical Density

It was desired to study the relationship between fluorescence of the dark germ extract and its optical density as determined spectrophotometrically. A sample of sound wheat germ was stored at 17 per cent moisture at 38° C. for approximately 30 days. During this period, browning occurred progressively in the sample from an initial stage of light tan to a final stage of dark mahogany color. Eight portions of wheat germ were withdrawn at various periods during this browning process. Then, 1 gm of each portion was extracted according to methods previously described and the optical density of each resulting extract was measured at 245 mu using B_2 filters and fluorescein as the standard. Fluorescence of each of these extracts was also measured. The optical density was then plotted versus the fluorescence intensity for each of the eight sample extracts. These data are plotted in Fig. 5 and show that relationship between fluorescence and optical density is a linear one. Additional evidence on the probable identity of the two factors is presented in later studies.

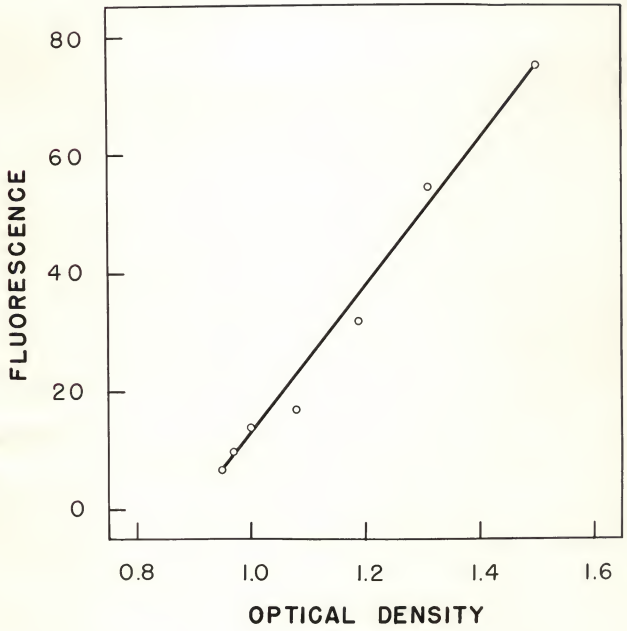

Fig. 5. Relationship between fluorescence of 0.2 N HCl extracts of browned germ and optical density.

Chromatographic Separation of the Dark Pigment from Aqueous Extracts of Brown Germ

Experiments were conducted to determine whether the brown colored substance could be separated by chromatography from the other constituents present in the brown aqueous wheat germ extract. The pigment appeared to be strongly adsorbed by such materials as magnesium oxide, Supercel, activated alumina, and Florisil (Fuller's earth preparation). The pigment was so strongly held by magnesium oxide and alumina that many washings with various solvents failed to release it. The pigment, however, could be released from Florisil by removing that portion of adsorbant containing the pigment from the column and allowing the adsorbant to stand in 0.2N HCl for several hours. During this time, the pigment slowly dissolved from the adsorbant.

The method of separating the dark pigment was essentially as follows. The 0.2N HCl extract obtained from browned wheat germ was freed of all proteinaceous materials by successively shaking it with chloroform. When these substances were removed, a clear amber colored liquid remained. A glass column, approximately 10 inches by 0.5 inches was then packed with Florisil. The extract was poured onto the column and a slight suction was applied at the bottom using a thick walled suction flask and an aspirator. Upon elution with 0.2N HCl, the components in the extract appeared to separate on the column into two distinct bands, a yellow flavone pigment in the lower region and the dark pigment in the upper

region. A portion of the yellow pigment was collected as it came through the column. It exhibited the absorption spectrum shown in Fig. 6. After this yellow pigment fraction was collected, the column was washed continuously with 0.2N HCl until no more of the yellow pigment could be detected spectrophotometrically in the washings.

The brown pigment on the column moved very little during this washing process. Then, the portion of the column containing the brown pigment was removed and added to some 0.2N HCl solution. The brown pigment eventually dissolved from the Florisil into the acid solution. The absorption spectra of this pigment is shown in Fig. 6. The yellow pigment is apparently a naturally occurring flavone as postulated by Markley and Bailey (22). Both the yellow and the brown substances exhibited fluorescence.

Fluorometric Method for Germ-damaged Wheat

For detecting differences between germ-damaged and sound wheat kernels, fluorometry proved to be more sensitive than spectrophotometry. Although the spectrophotometer was able to detect differences in the browning of wheat germ, the instrument was unable to pick up differences of any magnitude in extracts of ground "sick" wheat, Figs. 1 and 5. It is for this reason that the fluorometer rather than the spectrophotometer was used for measuring differences in ground wheat meal extracts.

The procedure for making these fluorometric determinations

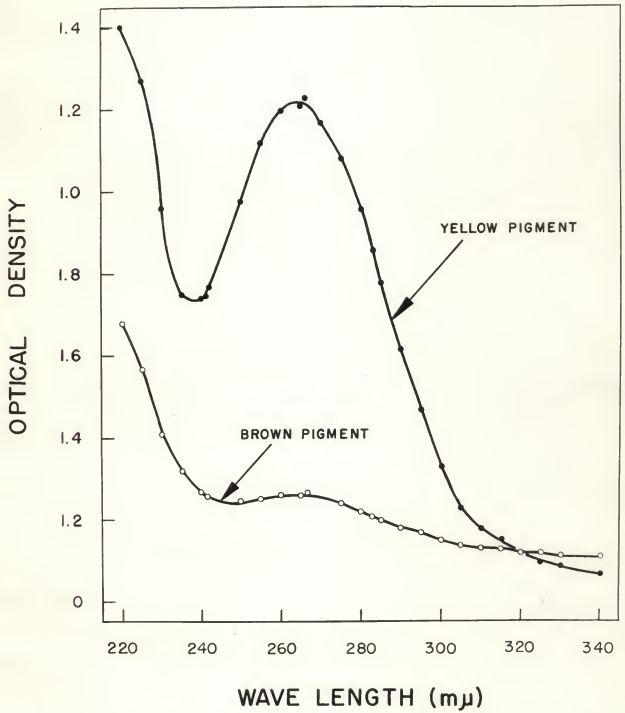

Fig. 6. Absorption spectra of brown and yellow pigments separated chromatographically from the 0.2 N HCl extracts of browned wheat germ.

was as follows. Only whole intact kernels were used. The sample of wheat studied was first cleaned of all chaff, hulls, and fragments. This sample was then ground in the Wiley mill using No. 30 screen. Three 6 gm samples of the ground wheat were weighed into Erlenmeyer flasks, 125 ml. Twenty ml of 0.2N HCl were added to each sample and extraction was carried on for 1 hour. The mixture was swirled once every 15 minutes. At the end of 1 hour, the mixture was centrifuged at 1500 rpm for five minutes and then filtered through Whatman No. 1 filter paper into a large test tube. Then, 5 ml of chloroform were added and the resulting mixture was shaken vigorously for 1 minute. (Much difficulty had previously been encountered in clarifying filtered extracts of ground whole wheat kernels. The extracts were quite cloudy, especially in the case of sound kernels, due probably to proteins and starches held in suspension. This condition of cloudiness was greatly remedied, however, by using a mild protein precipitant as employed by Sevag, Lackman and Smolens (26). With this method, the protein is denatured and rendered insoluble by shaking with chloroform.) The mixture containing the chloroform and wheat extract was centrifuged once more for approximately 10 minutes at 1500 rpm and then decanted. The precipitated protein remained at the bottom of the centrifuge tube together with the chloroform in the form of a gel. The extract resulting from this treatment with chloroform was fairly clear. This solution was then placed in the Coleman photoelectric fluorometer with B_2 filters and read without dilution, using fluorescein as the standard set at 60 and 0.2N HCl as the blank.

Alternatively, a 1 ml aliquot was diluted to 50 ml in a volumetric flask with 0.2N HCl and read in the fluorometer using the B_1 filters. According to Willard et. al. (34), the intensity of the fluorescent light for very low concentration of fluorescent substances, free of color and cloudiness, is proportional to the concentration of the substance. The B_1 filters were found best suited for this determination since the diluted extracts were completely free of color and cloudiness.

Establishing Linearity of Fluorescence

To determine whether the fluorescence of the substance in germ-damaged wheat was linear with increase in concentration over a greater part of the fluorometer scale, the following experiment was carried out. One germ-damaged sample of wheat was segregated completely into two portions, sound kernels and germ-damaged kernels. Each portion was ground in the Wiley mill. Six samples of 6 gms each were prepared from the two portions of ground wheat meal by mixing them proportionately to contain 0, 20, 40, 60, 80, and 100 per cent germ-damaged wheat by weight. The fluorescence of extracts prepared from these six samples was then measured and plotted versus the per cent of damaged wheat present in each sample. Fig. 7 shows that the fluorescence of the aqueous extracts of ground whole wheat increases linearly with increase in damaged grain in the wheat sample. This indicates strongly that the fluorescence technique provides a direct measure of germ deterioration

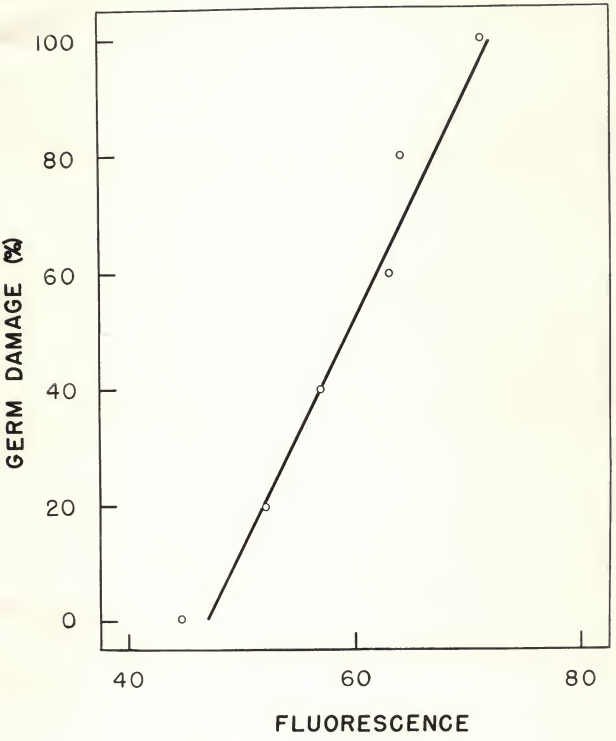

Fig. 7. Relationship between fluorescence of extracts and percentage of germ-damaged wheat in a sample.

in a given sample.

Fluorometric Characteristics of Extracts of Germ and Non-germ Portions of Damaged and Sound Wheat

It was of interest to determine to what extent the germ end of the damaged wheat kernel contributed to the total fluorescence as compared with the portion of the kernel containing the endosperm. From a sample of wheat graded 60 per cent germ-damaged, 6 gm of kernels having dark germs and 6 gm of kernels having sound yellow germs were segregated. The germ ends were sliced from all kernels in these two samples using a razor blade. (The germ portion contributed approximately 10 per cent of the weight of the sample of grain.) These dissected portions, the germ and non-germ, were ground in the Wiley mill and were then extracted for 1 hour using 0.2N HCl as solvent, keeping the sample to solvent ratio constant with 10 ml and 50 ml volumes of solvent for the germ and non-germ portions, respectively. At the end of 1 hour, the mixtures were centrifuged, decanted, the supernatant liquid was clarified, and its fluorescence was measured. The results are shown in Table 4. These data show that the greatest part of the fluorescence originates from the germ end of the kernel and increases in this portion considerably upon deterioration.

Table 4. Fluorometric characteristics of germ and non-germ portions of dissected germ-damaged and sound kernels.

	Germ-damaged kernels	Sound kernels
Weight of germ portion	0.71 gm	0.71 gm
Weight of non-germ portion	3.55 gm	3.55 gm
Fluorescence of germ portion	132.4 units/gm	25.6 units/gm
Fluorescence of non-germ portion	6.9 units/gm	3.9 units/gm

Correlation of Commercial Grain Inspection with the Fluorometric Test

To investigate the relationship between percentage of "sick" wheat damage as graded by commercial inspectors and the fluorescence test, approximately 26 samples of commercial germ-damaged wheat, graded by Federal Grain Inspectors in Kansas City were analyzed for fluorescence according to the method described in the previous section on fluorometric method for ground whole wheat. Germination tests were made by the Kansas State College Seed Testing Laboratory. The fluorescence values obtained on the 26 samples of germ-damaged wheat are listed in Table 5 along with the percentage germination of each. The correlation coefficients for the two sets of data, including fluorescence versus percentage germ-damage and fluorescence versus percentage germination, were found to be .748 and .775, respectively. These values are significant at the 1 per cent confidence level and show that a linear relation-

Table 5. Fluorescence of 0.2N HCl extracts and viability of germ damaged wheat graded at Federal Grain Inspection Office at Kansas City.

Graded damage (%)	Fluorescence	Germination (%)
5.0	34.0	78
7.0	37.7	83
9.0	34.0	48
12.0	39.4	50
15.0	50.5	63
16.5	50.0	57
17.0	38.4	34
17.0	36.2	39
18.0	43.0	37
20.0	40.1	56
22.0	42.0	55
22.0	30.0	63
24.0	48.8	65
25.0	60.7	27
26.0	50.0	44
28.0	51.0	31
36.0	62.0	0
40.0	56.0	16
40.0	42.7	32
50.0	61.0	13
63.0	54.0	10
64.0	61.5	0
68.0	55.5	4
70.0	58.0	8
76.0	62.0	1
80.0	59.7	17

ship exists between the two sets of data.

Fluorometric Studies of Different Classes and Varieties of Sound Wheat from Various Locations

Evidence obtained in this work shows that fluorescence of the extracts of wheat kernels increases with increase in germ damage. Extracts of sound wheat exhibit a normal fluorescence,

Table 6. Fluorescence of sound wheats.

Varieties	Date of harvest	Protein (%)	Ash (%)	Test wt. (lbs.)	Fluorescence
Composites from Wooster, Ohio					
Clarkan	1947	10.6	1.82	61.7	26.1
Kitt-ki	1947	11.0	1.77	60.7	26.0
Kawvale	1947	10.1	1.71	59.8	26.0
Kharkof	1947	10.2	1.73	60.2	23.3
American Banner	1947	9.5	1.71	58.5	25.0
Trumbull	1947	10.3	1.76	59.7	26.0
Fairfield	1947	9.3	1.69	58.8	27.0
Wabash	1947	10.1	1.67	60.7	24.7
Black Hawk	1947	11.3	1.76	60.8	30.0
Thorne	1947	9.8	1.74	58.5	26.0
Pacific wheats, Lind and Pullman, composites					
Triplet	1949	11.2	1.42	64.0	26.7
Rio	1949	12.3	1.55	64.2	27.8
Golden	1949	10.2	1.50	61.7	27.1
Hymar	1949	10.7	1.55	62.0	25.3
Rex	1949	11.9	1.63	63.0	27.0
Elgin	1949	10.7	1.47	63.0	26.0
Orfed	1949	11.0	1.53	64.0	26.6
Commanche	1949	12.9	1.54	64.0	26.2
Chiefkan	1949	11.3	1.54	67.4	24.3
Brevor	1949	10.7	1.43	64.0	26.7
Idaed	1949	13.1	1.47	62.0	25.1
Federation	1949	12.2	1.42	60.0	27.1
Marfed	1949	12.2	1.42	60.0	27.0

Table 6 (cont.)

Varieties	Date of harvest	Protein (%)	Ash (%)	Test wt. (lbs.)	Fluorescence
North Dakota wheats, Hard Red Spring					
Stewart Durum	1949				27.8
Mida	1949	15.6		61.9	27.0
Kansas State College Hard Red Winter					
Comanche	1952	11.4	1.79	60.2	23.7
Kiowa	1952	11.2	1.73	62.1	23.5
Pawnee	1952	11.0	1.57	61.5	22.5
Laude Environment Series Location: Manhattan, Kans.					
Pawnee	1952	10.7	1.65	62.1	27.3
Comanche	1952	10.6	1.76	61.0	31.0
Wichita	1952	9.9	1.67	63.0	30.0
Ponca	1952	10.7	1.73	61.9	31.0
Kiowa	1952	11.1	1.70	63.0	30.0
Location: Tribune, Kans.					
Pawnee	1952	16.8	1.73	54.9	32.5
Comanche	1952	17.0	1.80	53.8	32.5
Wichita	1952	15.7	1.70	59.0	30.0
Ponca	1952	16.7	1.80	54.0	31.7
Kiowa	1952	16.8	1.69	55.5	33.5
Red Chief	1952	16.0	1.66	58.0	32.0

Table 6 (cont.)

Varieties	Date of harvest	Protein (%)	Ash (%)	Test wt. (lbs.)	Fluorescence
Laude Environment Series (cont.)					
Location: Kingman, Kans.					
Pawnee	1952	12.6	1.34	61.6	29.0
Comanche	1952	13.7	1.51	60.5	29.0
Wichita	1952				27.5
Ponca	1952	12.9	1.44	62.0	28.0
Kiowa	1952	13.2	1.39	63.3	26.0
Red Chief	1952	13.0	1.45	64.0	27.5
Location: Belleville, Kans.					
Pawnee	1952	11.2	1.83	61.8	27.0
Comanche	1952	10.9	1.86	61.0	31.5
Wichita	1952	10.8	1.88	63.5	30.0
Ponca	1952	11.2	1.91	61.5	30.5
Kiowa	1952	11.1	1.82	63.0	31.0
Red Chief	1952	11.1	1.82	64.2	27.5
Location: Hays, Kans.					
Pawnee	1952	13.6	1.59	60.7	31.5
Comanche	1952	12.1	1.70	60.6	34.0
Wichita	1952	13.0	1.66	63.2	31.5
Ponca	1952	12.9	1.66	60.2	35.0
Kiowa	1952	12.5	1.64	62.0	30.5
Red Chief	1952	12.6	1.63	62.0	31.2

Table 6 (cont.)

Varieties	Date of harvest	Protein (%)	Ash (%)	Test wt. (lbs.)	Fluorescence
Laude Environment Series (cont.)					
Location: Hutchinson, Kans.					
Pawnee	1952	10.0	1.50	62.6	32.0
Comanche	1952	11.7	1.55	60.6	30.2
Wichita	1952	10.8	1.61	64.0	31.2
Ponca	1952	11.3	1.54	62.0	32.3
Kiowa	1952	11.6	1.51	62.1	30.5
Red Chief	1952	11.0	1.50	64.5	31.7
Location: Dodge, Kansas					
Pawnee	1952	15.8	1.52	61.0	33.2
Comanche	1952	15.9	1.63	60.4	32.5
Wichita	1952	15.0	1.57	63.0	30.0
Ponca	1952	14.9	1.64	61.2	2.5
Kiowa	1952	15.5	1.52	62.0	25.5
Red Chief	1952	14.9	1.54	63.2	26.7
Location: Garden City, Kans.					
Pawnee	1952	12.1	1.44	60.0	29.5
Comanche	1952	12.5	1.57	59.1	30.0
Wichita	1952	11.3	1.53	61.1	27.0
Ponca	1952	12.1	1.53	59.1	31.1
Kiowa	1952	12.8	1.50	60.6	28.0
Red Chief	1952	13.4	1.51	61.5	30.0

Table 6 (concl.)

Varieties	Date of harvest	Protein (%)	Ash (%)	Test wt. (lbs.)	Fluorescence
Laude Environment Series (cont.)					
Location: Colby, Kansas					
Pawnee	1952	13.0	1.75	59.0	30.0
Comanche	1952	13.4	1.90	58.0	32.5
Wichita	1952	12.2	1.79	61.0	29.0
Ponca	1952	12.8	1.80	59.0	32.5
Kiowa	1952	14.2	1.71	60.0	29.7
Red Chief	1952	12.7	1.76	60.0	29.5
Location: Manhatto, Kans.					
Pawnee	1952	16.1	1.60	54.0	24.5
Comanche	1952	16.1	1.77	53.5	29.3
Wichita	1952	15.0	1.73	57.6	25.5
Ponca	1952	16.8	1.82	54.2	30.7
Kiowa	1952	12.3	1.74	56.0	30.0
Red Chief	1952	14.5	1.60	59.0	30.0
Location: Thayer, Kans.					
Pawnee	1952	8.8	1.51	61.5	30.0
Comanche	1952	8.8	1.62	60.0	30.0
Wichita	1952	8.9	1.64	63.5	30.5
Ponca	1952	9.0	1.64	61.0	31.0
Kiowa	1952	9.0	1.53	62.5	30.0

but to less extent than extracts of germ-damaged wheat. The fluorescence of numerous samples of several classes and varieties of sound wheats was measured in order to determine this range of normal fluorescence. These data, together with information concerning protein content, ash content, and test weight, are listed in Table 6. The variability of fluorescence of 0.2N HCl extracts prepared from these wheat samples was found to be quite small. Fluorescence appears to have no relationship to protein content, ash, test weight or source of the sample.

DISCUSSION AND CONCLUSIONS

These studies are primarily of an exploratory nature but they indicate that a number of variables are probably involved in the deterioration known as "sick" or germ-damaged wheat.

A sample of germ containing 17 per cent moisture, stored at 25° C. browned after 23 days in storage. The 17 per cent moisture sample stored at 38° C. browned after 13 days. After 35 days in storage at 38° C. and 17 per cent moisture, the sample contained visible mold growth. Thus, it appears that browning of germ can occur in the absence of molds but their presence may increase the rate of browning. The chief factors which influence the rate of this browning phenomenon seem to be moisture and temperature. The fatty acid and reducing sugar increases which were found to occur in germ during storage are due,

probably, to molds. Many fungi are vigorous hydrolytic and lipolytic agents (12).

On the basis of the constituents present in the wheat germ, one may speculate concerning the nature of the brown product formed as a result of deterioration. Approximately 20 per cent of the germ is composed of sugars, chiefly sucrose (42 per cent) and raffinose (59 per cent) (10). At temperatures and humidities above normal, these sugars may react with the proteins and/or protein degradation products present in the germ to produce dark pigments. Similar types of browning occur in a variety of foodstuffs such as dried fruits, vegetables and dried egg products.

The use of wheat germ in the experimental work of this problem has advantages over the use of whole wheat kernels in that greater differences in browning can be detected both spectrophotometrically and fluorometrically. The absorption spectra and fluorescence obtained from ground whole wheat appear to be due almost entirely to constituents in the germ of the wheat. The absorption curves obtained from both ground wheat meal and dissected ground germ are strikingly similar in shape and trend. Only a small portion of germ as compared to that of wheat meal is needed to produce virtually the same spectral curve having the same shape with wider bands and more distinct differences.

The study of fluorescence of the extracts of germ and non-germ dissected portions shows that almost all of the increase in fluorescence during deterioration occurs in the germ of the

wheat kernel. Extracts of the endosperm or non-germ portion of germ-damaged kernels exhibit very little change in optical density and fluorescence except in the advanced stages of deterioration when some of the dark pigment from the germ apparantly diffuses into the endosperm of the kernel. As shown in Table 4, the dissected non-germ portion of the germ-damaged wheat kernels exhibits greater fluorescence than the non-germ portion of the sound kernel due to this diffusion of the dark substance into the endosperm.

The development of fluorescence in the browned wheat germ extracts parallels the increase in light absorption of the extracts at 245 mu. Thus, the fluorescing material appears to be the same as the substance responsible for the light absorption in the extracts, that is, the browning product. This conclusion is supported by the approximately linear relation, Fig. 5, that exists in the brown aqueous germ extract between absorption (optical density) and fluorescence.

The absorption spectrum of the brown pigment obtained by chromatographic separation showed considerable reduction in absorption peaks at 270 mu as compared to the original crude aqueous extracts of darkened wheat germ. Strong light absorption occurred only at low wavelengths and decreased with increase in wavelength, Fig. 6. Two substances of different color, responsible for the absorption, were separated by this method. They are the natural flavone pigment and the dark product formed as a result of germ deterioration.

The study of different classes and varieties of sound wheat shows that the range of normal fluorescence of their aqueous acid extracts, in contrast to the condition in germ-damaged grain, is quite narrow with a mean value around 28 for all samples examined. The reason for this low and fairly constant fluorescence intensity could be due not only to the fact that no germ deterioration existed but also because the pH of the extraction medium is low. At this pH, the fluorescence of the natural water soluble pigments and vitamins present in the extract is at a minimum. It is of interest to note that the fluorescence of sound wheat extracts remains fairly constant regardless of variety, protein content, location where grown, and other variables. for example, different soft red winter varieties grown in Wooster, Ohio, such as Clarkan, Minturki, and Thorne have a fluorescence value of 26.0. In this group of three varieties, there are wheats having protein contents ranging from 11.0 per cent for Minturki to 9.8 per cent for Thorne. The Wichita hard red winter variety grown in widely different locations of Kansas such as Belleville, Hays, and Dodge has a fairly constant fluorescence value ranging from 30.0 to 31.5.

The correlation coefficients for the two sets of data, fluorescence versus percentage germ-damage and fluorescence versus percentage germination, are .748 and .775, respectively. These two correlations are not significantly different from each other, indicating that fluorescence is as reliable in estimating germination as for estimating germ damage. Upon examination,

however, one finds the actual range of the fluorescence values, from 34 to 62, too small for predicting differences in kernel damage and germination. Further tests, utilizing a larger number of samples, will have to be made in order to fully evaluate this fluorometric test for determining per cent damage in wheat.

One normally encounters erratic variation from linearity when comparing two such types of test, that is, those of the highly subjective type of which commercial inspection for percentage germ damage is an example with those of a more sensitive nature such as fluorometry and colorimetry. Variation probably occurs between percentage germ-damage and fluorescence for the following reason. Each kernel in a sample of wheat probably does not deteriorate at the same rate, that is, browning does not occur evenly in all kernels affected by this type of deterioration; consequently, in a sample of "sick" wheat, there may be present kernels having germs varying shades of color from light tan to an intense black. Furthermore, commercial grain may be a mixture of different lots of varying degrees of damage. According to grading inspectors, all kernels showing any noticeable change in color of the germ or any signs of deterioration are regarded without qualification simply as 100 per cent damaged, whether its germ is light tan, dark brown, or any intermediate shade. The measurement of the fluorescence of a sample of wheat on the other hand, takes these gradations of germ-damage into consideration by measuring the overall average of germ-damage. This was verified experimentally. The plot of fluores-

cence of germ-damaged wheat extracts versus the true per cent of germ-damaged wheat in the sample was shown to be a linear function when a sample of 100 per cent germ-damaged wheat was ground and mixed uniformly in varying known proportions with ground sound wheat. In this manner, the true per cent of germ-damaged wheat present in the sample was known. The extracts from these samples so prepared showed a linear increase in fluorescence with increase in per cent germ-damaged wheat present, Fig. 7.

For reasons enumerated, one would expect little if any correlation upon comparing the methods of the inspector with that of measuring the fluorescence of grain extracts. For example, taking an extreme case from Table 5, a sample graded as 25 per cent germ-damaged fluoresces at 60.7 while an 80 per cent germ-damaged sample fluoresces at 59.7. It seems probable, after consideration of these aspects, that the fluorescence method may offer a more quantitative evaluation of the amount of deterioration present in a sample of germ-damaged wheat than does commercial grading.

SUMMARY

A study of the colorimetric and fluorometric properties of extracts of "sick" or germ-damaged wheat was carried out with the object of devising a method for evaluating germ-damage in commercial grain more quantitative than that provided by

commercial grading techniques. The following observations were made:

1. Of numerous organic and inorganic extraction media investigated, 0.2N HCl was found best for preparing clear extracts of germ-damaged wheat. In aqueous extracts at pH values greater than 7.0, a yellow color due to a natural pigment in the wheat germ appeared which interfered with colorimetric and fluorometric measurements. Also, at a low pH, the fluorescence due to the naturally occurring vitamin riboflavin was inhibited.

2. The spectral absorption curve of 0.2N HCl extracts of normal wheat was characterized by a peak at 270 mu and an inflection at 325 mu both of which increased slightly when the grain became germ-damaged. The damaged wheat extract also showed a marked increase in fluorescence over that of the sound wheat extract.

3. Fresh granular commercial wheat germ was found to darken in storage as did the germ in intact wheat, particularly when temperature and moisture conditions were elevated. Such conditions produced increases in fat acidity and non-reducing sugars.

4. Extracts of brown germ showed absorption peaks at 270 and 325 mu and a corresponding increase in fluorescence. These changes were more pronounced but very similar to those obtained with commercial "sick" wheat. Absorption at 245 and 325 mu as well as fluorescence increased markedly with increasing storage time and stages of deterioration of germ.

5. Wheat germ dissected by hand from sound and germ-damaged kernels picked from common samples of commercial "sick" wheat yielded extracts which showed the same spectral and fluorescence characteristics as those obtained from the stored granular germ. The same increase in optical density at 245 and 325 mu and in fluorescence, due to the "sick" condition, appeared.

6. The dark pigment in the germ of the "sick" wheat and in the browned germ of laboratory storage appears to be responsible for the increase in light absorption and fluorescence. It is suggested that this pigment is the product of a sugar-amino acid and/or protein condensation, better known as the "browning" phenomena.

7. Fluorescence of 0.2N HCl extracts of samples of germ-damaged commercial wheat was correlated with percentage of damage present as determined by grain grading inspectors. The relationship between these two variables was linear but the range of fluorescence obtained was too small for predicting accurately the percentage of germ-damage in a sample.

8. The normal range of fluorescence of numerous samples of several classes and varieties of sound wheats was found to be quite narrow. This is true regardless of the protein content, test weight, ash, and source of the grain.

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LITERATURE CITED

(1) American Association of Cereal Chemists
Cereal Laboratory Methods. 5th ed. St. Paul, Minnesota: American Association of Cereal Chemists. 117-119. 1947.

(2) Bailey, C. H.
The constituents of wheat and wheat products. American Chemical Society Monograph Series. New York: Reinhold Publishing Corporation, 168 p, 1944.

(3) Barnes, H. M., and C. W. Kaufman
Industrial aspects of the browning reaction. Ind. and Engr. Chem. 39:1167-1170. 1947.

(4) Burkholder, P. R., and I. McVeigh
Proceedings of the National Academy of Science. U. S. 28:440. 1943.

(5) Carter, E. P., and G. Y. Young
Effect of moisture content, temperature, and length of storage on the development of "sick" wheat in sealed containers. Cer. Chem. 22: 418. 1945.

(6) Carter, E. P., and G. Y. Young
Factors producing "sick" wheat in storage. Grain. December, 1947.

(7) Connor, R. T., and G. J. Straub
The thiamine and riboflavin contents of wheat and corn. Cer. Chem. 18(5)671-677. 1941.

(8) Coulter, C. B., F. M. Stone, and E. A. Kabat
The structure of the ultraviolet absorption spectra of certain proteins and amino acids. J. Gen. Physiol. 19:739. 1936.

(9) Danehy, J. P., and W. P. Pigman
Reaction between carbohydrates and proteins or amino acids. Quartermaster General (Research and Development Branch). 1945.

(10) DuBois, M.
Water soluble carbohydrates of the wheat germ. M. S. Thesis. University of Minnesota. 70 p. 1951.

(11) Frankel, M., and A. Katchalsky
Interaction of aldoses with alpha amino acids or peptides. Biochem. Jour. 35:1028-1033. 1941.

(12) Goodman, J. J., and C. M. Christensen
Grain storage studies. XI. Lipolytic activity of fungi isolated from stored corn. Cer. Chem. 29(4)299-308. 1952.

(13) Gortner, R. A.
Outlines of Biochemistry. 3rd ed. New York: John Wiley and Sons, Inc. 32-37. 1950.

(14) Greenstein, J. P.
Advances in Protein Chemistry. Vol. I. 222 p. New York: Academic Press Inc. 1944.

(15) Hannan, R. S., and C. H. Lea
Reaction between glucose and the terminal amino group. Nature. 168:744-745. 1951.

(16) Hoffer, A., W. F. Geddes, and A. W. Alcock
A rapid method for the determination of riboflavin in wheat and wheat products. Cer. Chem. 21:524. 1944.

(17) Johnson, T., and W. A. F. Hagborg
Melanism in wheat induced by high temperature and humidity. Can. Jour. Research. C22:7-10. 1944.

(18) Joslyn, M. A.
Color retention in fruit products. Ind. Engr. Chem. 33:308-314. 1941.

(19) Karrer, P., and H. Fritzsche
Konstitution and Fluoresbenz bei Flavinen. Helv. Chim. Acta. 19:481. 1936.

(20) Maillard, L. C.
Action des acids amines sur les sucres; formation des melanoidines par voie methodique. Compt. Rend. 154:66-68. 1912. (Original paper not seen.)

(21) Milner, M., C. M. Christensen, and W. F. Geddes
Grain storage studies. V. Chemical and Microbiological Studies on "sick" wheat. Cer. Chem. 24:23-38. 1947.

(22) Markley, M. C., and C. H. Bailey
The pigments of the dilute alcohol or acetone extract of whole wheat meal. Cer. Chem. 12(1)40-48. 1935.

(23) Mohammad, A., H. Fraenkel-Conrat, and H. S. Olcott
The "browning" reaction of proteins with glucose. Arch. Biochem. 24:157-178. 1949.

(24) Mrak, E. M., and G. F. Stewart
Advances in Food Research. Vol. I. New York: Academic Press Inc. 1948.

(25) Schultz, A. S., L. Atkin, and C. N. Frey
The vitamin B_1 content of wheat, flour, and bread. Cer. Chem. 16(5)643-648. 1939.

(26) Sevag, M., D. B. Lackman, and J. Smolens
The isolation of the components of streptococcal nucleoproteins in serologically active form. J. Biol. Chem. 124:425. 1938.

(27) Spies, J. R., and D. C. Chambers
Spectrophotometric analysis of amino acids and peptides with their copper salts. J. Biol. Chem. 191(2)787-797. 1951.

(28) Spies, J. R.
An ultraviolet spectrophotometric micromethod for studying protein hydrolysis. J. Biol. Chem. 195(1)65-74. 1952.

(29) Swanson, C. O.
Some factors involved in damage to wheat quality. Cer. Chem. 11:173-199. 1934.

(30) Stadtman, E. R.
Advances in Food Research. Vol. I. New York: Academic Press Inc. 1948.

(31) Tarassuk, N. R., and W. D. Simonson
Browning and the fluorescence of milk. Food Tech. 4:88-92. 1950.

(32) Tervet, I. W.
The influence of fungi on storage, on seed viability and seedling vigor of soybeans. Phytopathology. 35:3-15. 1945.

(33) Thomas, R. C.
The role of certain fungi in the "sick" wheat problem. Ohio Agri. Expt. Sta. Bimonthly Bull. 22:43-45. 1937.

(34) Willard, H. H., L. L. Merritt, and J. A. Dean
Instrumental Methods of Analysis. 2nd ed. New York: D. Van Nostrand Co., Inc. 23-26. 1951.

(35) Weast, C. A., and G. McKinney
Non-enzymatic darkening of fruits and fruit products. Ind. and Eng. Chem. 33:1409-1412. 1941.

(36) Wolfrom, M. L., R. D. Schuetz, and L. F. Cavalieri
Chemical interaction of amino compounds and sugars III. The conversion of D- glucose to 5(hydroxymethyl)-2-furaldehyde. Jour. Amer. Chem. Soc. 70:514-517. 1948.

(37) Wolfrom, M. L., R. D. Schuetz, and L. F. Cavalieri
Chemical interaction of amino compounds and sugars. Significance of furan derivatives in color formation. Jour. Amer. Chem. Soc. 71: 3518-3523. 1949.

(38) Zeleny, L.
Chemical and nutritive changes in stored grain. Trans. Amer. Assoc. Cer. Chem. 6(2)112-125. 1948.

APPENDIX

SUGGESTIONS FOR FUTURE RESEARCH

An important step which will aid in solving the "sick" wheat problem is the development of a method to determine accurately deterioration in wheat. Fluorometry offers a possible means. One of the weaknesses of the fluorometric test for determining per cent germ-damage in wheat, however, is its restriction to a rather narrow range. For example, for samples which are 5 to 80 per cent germ-damaged, there is a corresponding fluorescence range of only 34 to 62 units.

An investigation should be made in an effort either to broaden this fluorescence range or to increase the sensitivity of measurement within the range. This might be accomplished by using ultraviolet light filters other than the ones used in this work. The brown deterioration product may have stronger fluorescent properties at lower wavelengths than those used in this study. Another way one may be able to broaden this range is to inhibit the fluorescence arising from the natural pigments and vitamins, keeping the fluorescence due to the dark pigment constant. This also might be accomplished through the use of the other light filters or with ultraviolet light sources of greater range.

Obviously, a need exists for a better method than that afforded by grain grading inspectors for evaluating the percentage of germ-damaged wheat in a sample. The darkened germ of "sick" wheat has a physical property different from that of

sound germ in that it is much more brittle and friable. Consequently, in the milling of germ-damaged wheat there would be a tendency for the brittle darkened germ to be pulverized by the rolls and pass into the flour stream. Baking tests made with this flour may show the deleterious effect due to the presence of this darkened germ. For example, with increase in deteriorated germ, the flour may yield a corresponding reduction in loaf volume. The presence of the dark germ in the flour could be detected by fluorometry and could be correlated with the baking qualities of the flour. The correlation between two such tests may be highly significant.

A more complete study of this dark deterioration product in the germ should be made using chromatographic techniques, preferably paper chromatography. This study is a prerequisite for the identification of the browning product and might provide a clue as to its origin. The separation of the substance could be made on filter paper with the aid of an ultraviolet light. If several distinct compounds are found, it would be important to determine the relative rate of production of the various kinds in "sick" wheat development.

CHEMICAL CHANGES IN WHEAT RELATED TO TERM DETERIORATION

by

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The need for an investigation of the "sick" wheat condition has occurred due to extensive losses of grain in recent years, as a result of this type of damage. "Sick" wheat is that type of deterioration in which the seed loses its viability and the germ darkens in color and becomes rancid. The present study of the colorimetric and fluorometric properties of extracts of "sick" or germ-damaged wheat was carried out with the objective of devising a method for evaluating germ-damage in commercial grain more quantitatively than that provided by commercial grading techniques.

Of numerous organic and inorganic media investigated for preparing clear extracts of germ-damaged wheat suitable for spectral analysis, 0.2N HCl was found best. In aqueous extracts at a pH greater than 7.0, a yellow color, due to a natural pigment in the wheat germ, interfered with colorimetric and fluorometric measurements. Another advantage of this medium of low pH was that the fluorescence due to the naturally occurring vitamin riboflavin was inhibited.

The spectral curve for 0.2N HCl extracts of normal wheat was characterized by a peak at 270 mu and an inflection at 325 mu, both of which increased slightly when the grain became germ-damaged. The damaged wheat extract also showed a marked increase in fluorescence over that of the sound wheat extract.

Fresh granular commercial wheat germ was found to darken in storage as did the germ in intact wheat, particularly when

temperature and moisture conditions were elevated. Such conditions produced increases in fat acidity and non-reducing sugars. Extracts of brown germ showed absorption peaks at 270 and 325 mu and a corresponding increase in fluorescence. These changes were more pronounced but very similar to those obtained with commercial "sick" wheat. Absorption at 245 and 325 mu and fluorescence increased markedly with increasing storage time and stages of deterioration of germ.

Wheat germ dissected by hand from sound and germ-damaged kernels taken from common samples of commercial "sick" wheat yielded extracts which showed the same spectral and fluorescence characteristics as those obtained from the stored granular germ. The same increase in optical density appeared at 245 and 325 mu as well as an increase in fluorescence due to the "sick" condition.

The dark pigment in the germ of the "sick" wheat and in the browned germ of laboratory storage appears to be responsible for the increase in light absorption and fluorescence. It is suggested that this pigment is the product of a sugar-amino acid and/or protein condensation, better known as the "browning" phenomena.

Fluorescence of 0.2 N HCl extracts of samples of germ-damaged commercial wheat was correlated with percentage of damage present as determined by grain grading inspectors. The relationship between these two variables was linear but the range

was too small for predicting accurately the percentage of germ-damage in a sample.

The range of low fluorescence of numerous normal samples of several classes and varieties of sound wheats grown in widely separated areas was found to be quite narrow. This is true regardless of the protein content, test weight, ash, and source of the grain.

Fluorometry, rather than colorimetry, was found to be a more sensitive test for detecting varying degrees of germ-damage in wheat. Although fluorometry may not be well correlated with percentage damage as determined by grain grading inspectors in commercial samples of wheat, it has been found useful in studying the rate of darkening in stored wheat germ and may prove to be valuable for studying the rate of germ deterioration in grain stored in the laboratory.